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Department of
Agricultural Chemistry

29th November 1961

Professor Arthur Kornberg,
Department of Biochemistry,
School of Medicine,
Stanford University,
Palo Alto,
California.

Dear Arthur,

Thank you for your letter of 16th November. I was pleased to hear that the materials had reached you safely.

Thank you for sending me the preprint of Mahler's paper. It is interesting to know that he has progressed so far. His results are not seriously in conflict with our own, but I wish that he would at least do us the honour of quoting our results correctly ! The figures given in Table 1 for our bulk yeast DNA are incorrect; I have pencilled in the correct values on his paper, which I am returning to you (Montague & Morton, Nature 187, 916 (1960)). One of our preparations of yeast DNA showed good agreement with the results of Vischer, et al. (J. biol. Chem. 177, 429 (1949)) viz.

	A	G	C	T	$\frac{A+T}{G+C}$	$\frac{A}{T}$	$\frac{G}{C}$	$\frac{A+G}{C+T}$	$\frac{A+C}{G+T}$
Montague & Morton	32.2	17.4	16.6	33.8	1.94	0.95	1.05	0.99	0.95
Vischer et al.	31.7	18.3	17.4	32.6	1.80	0.97	1.05	1.00	0.97

The first point requiring comment concerns the base ratio of bulk yeast DNA. You have the details of the procedure which Dan Montague and I used to prepare the bulk yeast DNA: this gave $\frac{A+T}{G+C} = 1.94$. Now that preparation was not chromatographed. Unfortunately, we had none of that material remaining, so I used a fraction from the same yeast, and passed it through 'Ecteola', to remove traces of RNA and some oligonucleotides present in it. Thus the sample which you analysed was not directly comparable to that which Dan Montague and I used. Your

figure of $\frac{A + T}{G + C} = 1.65$ therefore really is applicable to a major fraction of yeast DNA.

Mahler's figures (YLE) also refer to a fraction taken out from bulk yeast DNA. I have not claimed, nor do I believe, that butanol-lactate treatment extracts all yeast DNA. Mahler's figures for this extract (YLE I & II) of $\frac{A + T}{G + C} = 1.36$ and 1.46 only are useful in demonstrating that the DNA associated with cytochrome b_2 is different from the bulk material - a conclusion which we had reached in 1957. We shall work up bulk DNA from our dried yeast again and I shall let you have some. Your figure of 2.48 for bulk yeast DNA suggests that yeast is a good material for study of DNA.

Now, dealing with the cytochrome b_2-DNA:

(a) Discrepancy in Reaction with Polymerase

The polymerase appeared to work well with the DNA bound to the enzyme. We also know that pancreatic DN-ase acts with the cytochrome b_2-bound DNA. We must therefore assume that the native cytochrome b_2-DNA is an effective template for your enzyme. If this is so, loss of effectiveness must represent denaturation (of some kind).

The question thus arises as to the difference between the first and second batches of cytochrome b_2-DNA sent to you. You will recall that Montague and I found that about 85% of the material applied to 'Ecteola' eluted between 0.5 and 0.7 M NaCl: the remaining material was obviously highly-polymerised, contaminant bulk DNA. We therefore prefer to purify the DNA by passage through 'Ecteola'. Hence Prep. I which you received, and which reacted so well with your enzyme, was a chromatographed preparation in NaCl.

Prep. II, however, was prepared as you had suggested when I was in Palo Alto. As I mentioned in my letter, I had not attempted to remove contaminant DNA. Prep. II was obtained by (a) splitting the DNA from cytochrome b_2 with ammonium sulphate, (b) dialysing against phosphate buffer, and then (c) dialysing against sodium citrate buffer. I had thought that you would chromatograph it on DEAE-cellulose (or on 'Ecteola') so that the trace of cytochrome b_2, and contaminant bulk DNA would be removed. I am sorry that there has been misunderstanding on this point; we already knew that the Prep. II was not homogeneous. (It is possible that my hand-written letter did not reach you).

It seems to me that there may be two explanations of the failure of Prep. II to act as an effective template. (1). Prep. II is more "denatured", viz. unlike the DNA on the cytochrome b_2.

(2). The contaminant bulk DNA acts as an inhibitor of the reaction with small DNA molecules. I wonder whether you have tried bulk DNA as an inhibitor ?

(b) Discrepancy in End-Group Analysis

I was not prepared to trust phosphate analyses for our study, as I knew that a trace of diesterase activity could invalidate our results. I did not then know of the E. coli alkaline phosphatase. We therefore depended on the base analyses. However, I do recognise that the error in the base analysis could be fairly large. However, we are fairly certain about the haem analyses, and the phosphate and base analyses for the whole enzyme. These indicate 17 phosphate groups per haem (Mahler's analyses confirm this). One of these phosphates is associated with FMN.

Now, (a) the DNA sediments in a centrifugal field along with the haem; (b) after three recrystallisations, the proportion of DNA per haem is fairly constant. The molecular weight from sedimentation and diffusion is 172,000, indicating two haem groups per molecule, and hence 32-34 phosphate groups per molecule of enzyme. I have therefore believed that this is the maximum size of the DNA. If it is single-stranded, then the molecular weight should be about 12-15,000.

I can only suggest, therefore, that the small amount of contaminant bulk DNA may be responsible for finding a chain length of 100 residues.

(c) Discrepancy in Base Analyses

I would most certainly accept your base analyses for cytochrome b_2. I believe that the error in the hydrolysis and chromatography of bases is fairly high. We rarely get better than 94-96% recovery of bases. Mahler's figures $\frac{A + T}{G + C}$ of 2.12 and 2.29 are chiefly due to a higher cytosine value than we had obtained. The base ratio of 2.6 which Cyril Appleby and I obtained for whole enzyme is almost certainly in error due to the difficulties arising from the large amount of protein present. It is apparent, however, that any contamination with bulk DNA would tend to lower, rather than raise, the $\frac{A + T}{G + C}$ ratio.

Mahler's paper looks fairly convincing to me, but you are much better able than I am to judge whether the material could still be double-stranded. The attack by Bob Lehman's enzyme appears to be good evidence in favour of single-strandedness.

I now feel that we must repeat the phosphate end-group study on intact cytochrome b_2, i.e. on the material which is so effective as a template for your enzyme. I am still waiting for Worthington to send

me the bacterial alkaline phosphatase which we have ordered. Could you send me some spleen diesterase when you make your next preparation. I feel that it would be a good idea to do the analyses in both laboratories.

I am also attempting a further selective purification of the cytochrome $\underline{b}_2$-DNA. We now have adequate amounts of crystalline type II-cytochrome $\underline{b}_2$ which is free of DNA. I am allowing this DNA-free enzyme to re-combine with cytochrome $\underline{b}_2$-DNA purified by chromatography. Recombination is indicated by a specific crystal form. Thus I can use the cytochrome $\underline{b}_2$ to select out the specific DNA. This may enable us to get rid of any contamination. I shall let you know of progress.

Could you send us some of Bob Lehmann's enzyme with the spleen enzyme. Again, I think that it would be desirable to confirm Mahler's results on this point.

Addendum, 17/12/61

Dear Arthur, I was to leave tomorrow for U.K., but physical and mental pressures of last few weeks caught up on me and I landed here in Adelaide Hospital on Thursday night last. However, the medical report is good, the heart has suffered no serious damage & I can leave here on Wednesday & fly to Sydney on Friday to catch the ship. So all is well. The 4 weeks on the ship will put me right on top again.

This letter was not sent because I was awaiting the result of recombination expts. Please do not pass these results to Mahler or anyone else. I have been able to recombine the DNA-free enzyme & apparently select out from DNA mixtures a portion of the DNA, giving specific crystalline forms of the enzyme. These are being worked on now. Most of the sample of cyt. b_2-DNA sent to you is 'denatured' in the sense that it does not recombine to give the specific Type I-crystal. From thymus gland DNA, I have selected out a small amount material giving a beautiful new crystalline form - a cube  — one I have never seen before.

This confirms the specificity of b_2-DNA only for the true Type I crystal — non-crystalline material may carry other types of DNA molecules.

The potential of this finding is considerable, & I know that you will respect my confidence especially since Henry Mahler & [illegible] hard on our heels. My immediate suggestion is that we do as much work as possible with the DNA on the enzyme, & that I send you more cyt. b_2 (Type I). Can you use this?

Best wishes for Christmas & the New Year — Warmest regards, Ted